Please amend Claim 351 as follows:

351. (Twice amended) A method according to Claim 329 wherein said targeting ligand is a peptide comprising a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1).

REMARKS

Reconsideration of the present application in view of the above amendments and following remarks is requested respectfully.

Status of the Claims

Claims 100, 102, 103, 127, 194 to 200, 203, 210 to 228, 294 to 300, 303, 310 to 329, 331 to 337, and 347 to 356 are pending in the application. Claims 113, 115, 122, 124, 229 to 238, 245 to 248, 255 to 270, 277 to 280, 287 to 292, and 357 to 411 have been canceled, without prejudice. No claims have been added. Claim 351 has been amended.

The amendment clarifies that the targeting ligands are peptides which comprise the recited amino acid sequences. Support for this language may be found in the specification, for example, at page 67, lines 7 to 9, or page 73, lines 7 to 10. It is believed that this claim amendment, in conjunction with the cancellation of claims, fully addresses the rejection under 35 U.S.C. § 112, second paragraph.

Applicants thank the Examiner for indicating that the present application has an effective filing date of May 1, 1996, and for withdrawing the previously pending rejection under 35 U.S.C. § 103.

Summary of the Invention

The invention defined by the pending claims is directed to formulations for diagnostic or therapeutic use that comprises lipid vesicles encapsulating a fluorinated gas and bearing a targeting ligand, and methods for therapeutic delivery *in vivo* that comprise administering such formulations. Claims 100 and 127, the only two pending independent claims, both importantly recite that the targeting ligand *is covalently bound to the lipid vesicles via a* ***hydrophilic polymer linking group***, and *targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor*. These claims further clarify that the fluorinated gas in the vesicles is selected from the group consisting of perfluorocarbons and sulfur hexafluoride.

Rejection under Section 102(e)

Claims 100, 102, 127, 194, 203, 294, 303, and 320 to 338 stand rejected under 35 U.S.C. 102(e) as being anticipated by Lanza et al., U.S. Patent No. 5,989,520 ("Lanza").

As a preliminary matter, Applicants reiterate that Lanza '520 is not proper prior art to the instant application, because the application that contained the same disclosure as Lanza '520 was originally filed after the May 1, 1996 priority date of the instant application. Applicants recognize that Lanza '520 claims to be a continuation-in-part of application No. 08/488,743, now U.S. Patent No. 5,690,907 ("Lanza '907"). However, the disclosures of the two patents are not the same, with Lanza '520 containing additional disclosure that was not present in the original application that matured into Lanza '907 (which is why the parent of Lanza '520 was

a continuation-in-part, not a straight continuation, of the application that matured into Lanza '907). For example, Example 18 of Lanza '520, which is expressly relied on in the Office Action in making the rejection over Lanza '520, is **not present** in Lanza '907. Applicants respectfully submit that 35 U.S.C. § 102(e) requires that the disclosure relied upon by the Examiner must have been present in an application filed in the United States before the priority date of the instant application. Since the Lanza '907 patent, and not Lanza '520, contains the disclosure of the application that was filed prior to Applicants' May 1, 1996 priority date, Applicants will continue to address their comments to the Lanza '907 patent, rather than the Lanza '520 patent cited in the Office Action.

Summary of the Lanza '907 Patent

The Lanza '907 patent is generally directed to a method for ligand-based binding of lipid encapsulated particles to molecular epitopes on a surface comprising sequentially administering (a) a site-specific ligand activated with a biotin activating agent; (b) an avidin activating agent; and (c) lipid encapsulated particles activated with a biotin activating agent, whereby the ligand is conjugated to the particles through an avidin-biotin interaction so that the resulting conjugate is bound to the molecular epitopes. *See* Lanza '907 abstract. Perfluorcarbon emulsions may be encapsulated with the lipid particles, and the emulsions may generate gaseous vapors. *See* col. 6, lines 59 to 62. Lanza further describes both diagnostic and therapeutic applications for this system. *See* col. 7, lines 7 to 67.

Lanza '907 Does Not Anticipate Applicant's Claimed Invention

As stated in the Response submitted January 15, 2001, Applicants claims distinguish over the Lanza '907 patent by defining targeted lipid vesicles that encapsulate a *fluorinated gas*. Lanza does not describe any such entities. Lanza teaches the encapsulation of perfluorocarbon emulsions, such as perfluorotributylamine, perfluorodecalin, and the like (*see* Lanza '907, col. 6, liees 5 to 9). Although Lanza suggests that vapors may be evolved from these dense vapors (*see* col. 6, line 61) Lanza does ***not*** teach or suggest the use of even a single fluorinated gas. Lanza does not anticipate Applicants' claimed invention for this reason, if for no other.

Applicants' claims also distinguish over Lanza '907 by reciting that the vesicles bear a targeting ligand that is bound to the lipid vesicle *via a hydrophilic polymer linking group.* As described in the instant application, for example at pages 80 to 82, such lipid-polymer-targeting ligand entities may be in the form of, for example, compounds of the formula L-P-T, wherein L is a lipid, P is a hydrophilic polymer, and T is a targeting ligand. Preferred hydrophilic polymers include, for example, polyalkyleneoxides, polyvinyl alcohol, polyvinylpyrrolidones, polyacrylamides, polymethacrylamides, polyphosphazenes, poly(hydroxyalkylcarboxylic acids) and polyoxazolidines (*see* page 81, line 30 to page 82, line 1).

Lanza fails completely to teach, describe or suggest lipid vesicles in which a targeting ligand is bound to the lipid by a hyrophilic polymer, as defined in Applicants' claims. It is asserted in the Office Action that Lanza discloses using a polymerized lipid or a lipid with

ether or ester linked fatty acids. Applicants respectfully submit that any extrapolation from Lanza's statements regarding the lipids that may be used for encapsulating the perfluorocarbon emulsion, to the lipid-polymer-targeting ligand entities of the present invention, is a misinterpretation of Lanza's teachings. As discussed above, Lanza is clearly and unequivocally directed to the use of an avidin/biotin reaction to bind the targeting ligand to the liposome. In the passage cited in the Office Action, Lanza states

> In a specific example, the lipid encapsulated particles may be constituted by a perfluorocarbon emulsion, the emulsion particles having incorporated into their outer coating a *biotinylated lipid compatible moiety* such as . . . a lipid with ether or ester linked fatty acids or a polymerized lipid.

See Lanza '907, col. 5, lines 43 to 51 (emphasis added). Lanza is teaching in this passage that a perfluorocarbon emulsion may be encapsulated in a lipid vesicle, which includes a lipid compatible moiety, such as a lipid with an ester linked fatty acid, which has been "biotinylated." This is clearly ***not*** a description of a targeting ligand, which targets one of the cell or receptor types recited in Applicants' claims, bound to a lipid via a hydrophilic polymer. Applicants respectfully submit that Lanza does not anticipate Applicants' claims for this reason also.

Moreover, Lanza's tri-phasic, sequential administration procedure (*see* col. 4, lines 44 to 56) is clearly completely different than the methods of the present invention. In Lanza's method, a site-specific targeting ligand that has been activated with a biotin activating agent is administered separate from the biotinylated lipid particles, and an avidin/biotin interaction takes place within the patient, to bind the particles to the desired binding site. Thus,

Lanza's method does not comprise administering targeted lipid vesicles that are ***covalently bound*** to the targeting ligand, as recited in Applicants' claims.

In view of the foregoing, Applicants respectfully submit that Lanza '907 (like Lanza '520), does not disclose or suggest the invention defined by Applicants' claims. Accordingly, Applicants respectfully request that the rejection under Section 102 be withdrawn.

Rejection under Section 103(a)

All pending claims stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Porter, U.S. Patent No. 5,648,098 ("Porter"), in view of Lanza '520 and Konigsberg et al., U.S. Patent No. 5,258,499 ("Konigsberg") or Trubetskoy et al., Biochemica et Biophysica Acta 1131 (1992) 311-313 ("Trubetskoy), and Ginsberg, U.S. Patent No. 5,656,442 ("Ginsberg") and Siegel et al., U.S. Patent No. 5,695,460 ("Siegel"). Applicants respectfully traverse this rejection, on the grounds that the references, in any proper combination, fail to teach of suggest the formulations and methods of the present invention.

Porter, the primary reference relied upon in the Section 103 rejection, describes a method of treating thrombosis comprising administering a composition of "perfluorocarbon enhanced sonicated dextrose albumin microbubbles" (referred to hereinafter, as in Porter, as "PESDA microbubbles") and applying ultrasound to the site of the thrombus. *See e.g.*, col. 6, Example 1. It is conceded in the Office Action that Porter does not teach the use of a lipid vesicle. It must also be noted that the PESDA vesicles used by Porter also do not contain a bioactive agent, and Porter, in fact, *teaches away*, from use of the disclosed PESDA

microbubbles in combination with a bioactive agent, as recited in applicants' claims. *See, e.g.*, Porter's Abstract ("The methods and pharmaceutical composition of the invention exhibit thrombolytic properties similar to those of other thrombolytic agents such as urokinase and are less toxic and are clot specific in that they do not introduce a systemic lytic state to a said animal"). The PESDA microbubbles described by Porter also do not contain any targeting ligand, much less one directed to the cells or receptors recited in Applicants' claims. Of course, since Porter discloses neither lipid vesicles nor targeting ligands, Porter completely fails to suggest that a targeting ligand may be covalently bound to the lipid via a hydrophilic polymer linking group. It is clear, therefore, that Porter in no way teaches or discloses the formulations defined by Applicants' claims. Moreover, Porter does not describe *any* methods for the therapeutic delivery *in vivo* of a bioactive agent, since Porter's pharmaceutical compositions do not contain any bioactive agent (*see, e.g.*, col. 2, lines 21 to 26).

The deficiencies of Porter are said in the Office Action to be overcome by combination with the other cited references. The disclosure of Lanza '907 (and Lanza '520) are discussed above. It is said in the Office Action that Lanza "is primarily used to show that liposomes containing perfluorinated emulsions provide improved targeting specificity when they are attached to a targeting ligand." Porter is not directed to liposomes, however, and while Lanza may show increased target specificity, this increased specificity is said to be for the purpose of *identifying sites* within the patient, not for the purpose of *lysing thrombi*, which is the purpose to which Porter is directed. Thus, there is no suggestion in either of the references which would motivate one skilled in the art to modify the PESDA microbubbles used by Porter by using gas-

filled liposomes that bear a targeting ligand. In fact, Lanza emphasizes the desirability of using vesicles at extremely low blood concentration levels. *See* Lanza '907, col. 7, lines 17 to 20 and 36 to 40 ("the background contrast from lipid encapsulated particles in the blood is minimal"). Applicants respectfully submit that the skilled artisan would have no reason to believe that such low blood levels of vesicles would be sufficient to achieve the thrombolysis that is the subject of Porter. The skilled artisan would therefore have no motivation to modify Porter's vesicles using the methods described by Lanza, because there would be no reasonable expectation that such a modification would succeed. Applicants respectfully submit that the combination of the two references, as done in the Office Action, is improper for this reason also.

Moreover, even if Porter is combined with Lanza, the deficiencies of Lanza which were discussed with regard to the Section 102 rejection are still evident. Lanza does not teach or suggest methods involving a formulation comprising lipid vesicles bearing a targeting ligand that is bound to the lipid via a hydrophilic polymer linking group: to the contrary, Lanza describes a tri-phasic administration, in which the disclosed liposomes ansd targeting ligands are administered ***separately***. According to Lanza, the targeting ligand and the lipid vesicles may become bound together, *in vivo*, via an avidin/biotin complex. Thus, even if Lanza and Porter are improperly combined, as in the Office Action, one still fails to arrive at the methods and formulations defined by Applicants' claims.

These deficiencies are not remedied by further combination with Konigsberg. Konigsberg describes liposomes that have ligands covalently attached to the surface by coupling agents (linker molecules) such as SATA or SPDP, but contains no teaching or suggestion that a

hydrophilic polymer may be used for this purpose (*see, e.g.*, col. 4, lines 1 to 46). Similarly, although Trubetskoy may well teach the preparation of targeted cationic liposomes via an ionic bridge between cationic liposomes and a targeting moiety, the targeting ligand is neither *covalently linked*, nor is it linked *via a hydrophilic polymer linking group*, as recited in applicants claims. Ginsberg, which describes the KQAGDV targeting ligand, fails to teach or suggest linking of same to a gas-filled lipid vesicle, and Siegel, which is devoid of any teaching or suggestion regarding the use of targeting ligands, also fails to overcome the deficiencies of the previously discussed references.

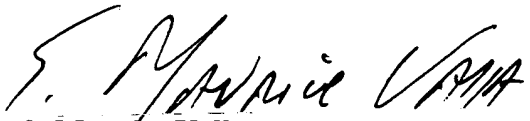
Any proper combination of these references does not, therefore, teach or suggest the methods and compositions defined by Applicant's claims. None of the references teach the use of hydrophilic polymer linking groups for covalently binding targeting ligands to lipid vesicles. Accordingly, Applicants respectfully request that the rejection under Section 103 be reconsidered and withdrawn.

CONCLUSION

Applicants believe that the foregoing constitutes a full and complete response to the Office Action of record. Accordingly, an early and favorable Action is requested respectfully.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."** Also attached is a copy of the claims Applicants believe to be pending after entry of the amendment, as requested by the Examiner.

Respectfully submitted,



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Date: **March 7, 2002**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 351 has been amended as follows:

351. (Twice amended) A method according to Claim 329 wherein said targeting ligand [comprises] is a peptide comprising a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1).

Claims 113, 115, 122, 124, 229 to 238, 245 to 248, 255 to 270, 277 to 280, 287 to 292, and 357 to 411 have been canceled, without prejudice.